

## Draft genome sequence of *Inquilinus limosus* strain MP06, a multidrug-resistant clinical isolate

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### Abstract

The bacterium, *Inquilinus limosus*, with its remarkable antimicrobial multiresistant profile, has increasingly been isolated in cystic fibrosis patients. We report draft genome sequence of a strain MP06, which is of considerable interest in elucidating the associated mechanisms of antibiotic resistance in this bacterium and for an insight about its persistence in airways of these patients.

**Key words:** *Inquilinus limosus*, cystic fibrosis, antimicrobial resistance,  $\beta$ -lactamases, multidrug resistance efflux pumps.

### Genome Announcement

*Inquilinus limosus* MP06 was the first isolate reported in Latin-American and was recovered from a chronically colonized pediatric cystic fibrosis patient (CFP) (Busquets *et al.*, 2013; Pino *et al.*, 2014). There have been a limited number of specimen isolations from other parts of the world (Bittar *et al.*, 2008; Hayes *et al.*, 2009; Salvador-García *et al.*, 2013). This typical mucoid isolate displays high levels of resistance to several antibiotic families, including colistin, nalidixic acid, chloramphenicol, fosfomicin, trimethoprim-sulfamethoxazole, nitrofurantoin, kanamycin, gentamicin, tobramycin, tetracyclines, and  $\beta$ -lactams even in their combination with  $\beta$ -lactamase inhibitors (except for carbapenems), but it remains susceptible to amikacin and ciprofloxacin (Pino *et al.*, 2014). Herein, we report the draft genome sequence of *I. limosus* strain MP06.

Total DNA was sequenced by a whole-genome shotgun (WGS) strategy using a 454 GS Titanium pyrosequencer at INDEAR (Santa Fe, Argentina). A total of 348,145 sequencing reads were assembled *de novo* (Newbler v2.6 using the -urt option), generating 1,186 high quality contigs covering 99.8% (6,934,542 bp) of the total predicted genome, with a mean G+C content of 69.6% and a 20x total average coverage. The codon bias (as depicted by GCUA 1.2 software, McInerney, 1998) showed that the

alternative start codon, GUG, is present in a high frequency of occurrence (Relative Synonymous Codon Usage - RSCU = 1.88), which is a bit expected for high G+C content.

Genome annotation was performed by using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) version 2.0 (Angiuoli *et al.*, 2008). The draft sequence consists of 7,170 genes, among them, 6,229 correspond to coding-DNA sequences (CDSs), 890 to pseudogenes, 47 to tRNA genes and 3 to rRNA regions.

As expected for a microorganism, wherein no experimental evidence of any extrachromosomal DNA presence by plasmid extraction or pulsed-field gel electrophoresis analysis with S1 endonuclease could be found, no sequence related to replication origins of plasmids, nor any marker for extrachromosomal elements could be detected. Chromosomally located putative genes involved in antibiotic and toxic compound resistance mechanisms were determined by the use of the Rapid Annotation using Subsystem Technology (RAST) annotation server (Aziz *et al.*, 2008). Multidrug resistance efflux pumps organized in different efflux systems, such as the CmeABC operon (*cmeA*, *cmeB*, *ATPase AAA Family*) and other genes arrangements of RND family, and MacA-MacB efflux system (*macA*, *macB*, *nodT*) of the ABC superfamily, were also found. The functionality of these efflux systems (Pino *et al.*, 2013a) might be taken as an explanation for the intrinsic resistance

of this species with the consequent negative impact on the therapeutic control of lung infections in CFPs.

Eleven genes could be assigned to penicillin-interacting proteins (including 7 PBPs) and four to putative  $\beta$ -lactamases, two of them have already demonstrated enzymatic activity, as previously reported (Pino *et al.*, 2013b; Pino *et al.*, 2014). Various resistance determinants toward arsenic, copper, cobalt, zinc and cadmium, and genes involved in biofilm formation were also found. Though not predicted in this *in silico* analysis, other factors like outer membrane low permeability may also influence the overall antimicrobial resistance.

The genome sequence of MP06 may provide a basis to understand the antibiotic resistance mechanisms and its ability to persist in CFPs' airways even under prolonged antimicrobial therapy.

Nucleotide sequence accession numbers: This WGS project has been deposited at DDBJ/EMBL/GenBank under the accession number JAXX000000000. The version described in this paper is version JAXX010000000.

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## References

- Angiuoli SV, Gussman A, Klimke W *et al.* (2008) Toward an on-line repository of Standard Operating Procedures (SOPs) for (meta) genomic annotation. *OMICS* 12:137-141.
- Aziz RK, Bartels D, Best AA *et al.* (2008) The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9:75.
- Bittar F, Leydier A, Bosdure E *et al.* (2008) *Inquilinus limosus* and cystic fibrosis. *Emerg Infect Dis* 14:993-995.
- Busquets NP, Baroni MR, Ochoteco MC *et al.* (2013) Bacterial isolates from respiratory samples of pediatric patients with cystic fibrosis and their distribution by ages. *Rev Argent Microbiol* 45:44-49.
- Hayes D Jr, Murphy BS, Kuhn RJ *et al.* (2009) Mucoid *Inquilinus limosus* in a young adult with cystic fibrosis. *Pediatr Pulmonol* 44:619-621.
- McInerney JO (1998) GCUA: general codon usage analysis. *Bioinformatics* 14:372-373.
- Pino M, Di Conza J, Gutkind G (2013a) Exploración de bombas de eflujo en un aislamiento clínico de *Inquilinus limosus*. *Rev Argent Microbiol* 1(Suppl):P-386.
- Pino M, Di Conza J, Gutkind G (2013b) A novel chromosomal class C  $\beta$ -lactamase in *Inquilinus limosus*. 53<sup>rd</sup> Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, USA, abstr. C1-1591.
- Pino M, Power P, Gutkind G *et al.* (2014) INQ-1, a chromosome-encoded AmpC beta-lactamase from *Inquilinus limosus*. *J Antimicrob Chemother* 69:560-562.
- Salvador-García C, Yagüe-Guirao G, Pastor-Vivero MD *et al.* (2013) Chronic colonization of *Inquilinus limosus* in a patient with cystic fibrosis: First report in Spain. *Enferm Infecc Microbiol Clin* 31:414-415.

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